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Dominant Lethal Effects of Subchronic Acrylamide Administration in the Male Long-Evans Rat

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Dominant lethal effects of subchronic acrylamide administration in the male Long–Evans rat

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Summary

Acrylamide, a widely used vinyl monomer, is well known as a neurotoxin but inactive as a mutagen in bacterial test systems. The experiments reported demonstrate that after subchronic oral dosing in the male rat, acrylamide induced significant elevations in both pre- and post-implantation loss following dominant lethal testing. These effects were seen at doses which failed to produce clinical or pathological evidence of neurotoxicity. In an accompanying cytogenetic study, no increase in chromosome aberrations was observed in spermatogonia or spermatocytes of treated animals. When spermatocytes from treated spermatogonial stem cells were analyzed, reciprocal translocations (4) were observed in the treated animals and not in the control, but the significance of this result still needs to be established.

Acrylamide is a reactive vinyl monomer widely used in the synthesis of polymers for a variety of industrial applications. The monomer is neurotoxic to both laboratory animals and man, producing a classical peripheral neuropathy and having specific influences in the striatal dopaminergic system (Tilson, 1981, for review). Although the compound is highly water-soluble, its neurotoxicity appears to be related to cumulative dose and dosing schedule. There is an inverse relationship between the dose and the length of the latent period from the start of treatment to the onset of effects.

Several authors have reported other toxic effects of acrylamide including testicular atrophy (Hashimoto et al., 1981; McCollister et al., 1964; Burek et al., 1980), clastogenic effects in bone marrow and spermatogonial cells (Shiraishi, 1978), and carcinogenesis in the mouse (Bull et al., 1984, 1984a). Acrylamide is not active in the Ames mutagenesis assay with or without added S9 metabolizing capacity (Lijinsky and Andrews, 1980; Bull et al., 1984). [^{14}C]Acrylamide binds to

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both proteins and nucleic acids in mice (Hashimoto and Aldridge, 1970; Carlson and Weaver, 1985); the role of biotransformation in its toxicity and the active moiety have not been established.

During the course of a study designed to describe the influence of acrylamide on male and female reproductive function, Zenick et al. (1985) observed infertility and post-implantation losses in untreated female rats mated to males drinking 100 ppm of acrylamide for 10–11 weeks. No effects were noted on testicular histology or sperm parameters in these males. This communication reports a more detailed examination of the potential dominant lethal effect of acrylamide in the Long–Evans rat.

Materials and methods

Monomeric acrylamide (CAS-79-06-1) (Eastman Kodak), was assayed as >99% pure by gas chromatography. Long–Evans rats of breeding age were obtained from Charles River (Portage, MI) and housed individually, the males in hanging steel cages and the females in polycarbonate boxes with Ab-Sorb-dri® bedding. The animal room was maintained at 70–74° F and 55% humidity, with a 12:12 light:dark cycle, lights on at 0700 h. Animals were given ad libitum access to Purina Lab Chow and distilled water containing acrylamide solutions as drinking water. Control animals received distilled water. Acrylamide solutions were prepared biweekly and administered from amber glass bottles. Animal weights and fluid consumption were monitored twice weekly.

The males were weight matched, assigned to groups and received 0, 15, 30 or 60 ppm of acrylamide in their drinking water for a total of 80 days. There were 11 males in the 60 ppm group; all other groups contained 10 males. At the end of 72 days, each male was housed overnight with two virgin females during which period the water bottle was withdrawn. The females were removed the following morning and subjected to vaginal lavage. Those observed to be sperm positive were designated at day 0 of gestation. The mating trials continued until each male had impregnated two females or for a maximum of 8 days.

The females were sacrificed on day 14 of gestation and examined for numbers of corpora lutea and for living and dead fetal implants. Fertility rates and percentages of pre- and post-implantation losses were calculated from these figures (see legend to Table 2). Data on fertility rates were analyzed by Chi-square statistics. The effects of acrylamide on pre- and post-implantation loss were analyzed using Kruskal–Wallis ANOVA with Mann–Whitney *U* Test for post-hoc comparisons. For each male, the fetal outcomes of the females he had successfully impregnated were averaged to yield a single score (i.e., the male was the unit for analysis).

After the completion of the breeding trials, half of the males in each group (6 males in the 60 ppm group) were sacrificed. Colchicine was injected 4 h prior to sacrifice. Testicular preparations were made by the Meredith method (Meredith, 1969) and slides stained with Giemsa. 50 spermatogonial metaphases and 50 spermatocyte diakinesis-metaphase I cells were analyzed on all but 3 males. 12 weeks later, the remaining males were sacrificed in order that reciprocal translocations, in the form of multivalent configurations, could be analyzed in spermatocytes that were derived from treated spermatogonial stem cells. In all but one case, 50 spermatocytes per male were analyzed.

Since sciatic nerve impairment is a documented landmark of acrylamide neurotoxicity (Tilson, 1981), it was important to note the incidence of this classical lesion in the presence of dominant lethality. Thus, for the males that were sacrificed after completion of breeding, the sacral, sciatic and tibial nerves with their terminal branches were dissected from each hind limb. From the right leg these nerves were placed in a mixture of 4% formalin and 1% glutaraldehyde (4FIG) and those from the left leg were placed in 10% buffered formalin. Formalin fixed nerves were processed, embedded in paraffin, sectioned at 5–6 microns and stained with hematoxylin and eosin as well as Holmes silver and Luxol fast blue. Nerves fixed in 4FIG were processed, embedded in plastic, sec-

tioned at 1–2 microns and stained with toluidine blue.

Results

No differences were observed between treated and control males for weight gain or fluid consumption. The acrylamide intake expressed as mg/kg/day or as cumulative mg/kg at initiation of mating, varied in a dose-responsive manner (Table 1).

The fertility rate was calculated as the percentage successfully impregnated of those females to which a male was given mating access. One male in each of the 0 and 25 ppm groups failed to mate with either of his 2 females. The fertility rate was comparable in all dose groups (Table 2).

There was a significant elevation in preimplantation loss only in females mated to the high dose males. Post-implantation loss was increased for males drinking both 30 and 60 ppm acrylamide. At the high dose the percentage was more than 6-fold that found in controls.

Preliminary cytogenetic analyses were undertaken by examining testicular preparations for half of the males in each dose group immediately after the termination of the breeding studies and from the remainder at 12 weeks post-treatment. No increase in aberrations was seen in the treated animals at the first sacrifice. In the animals sacrificed 12 weeks later, a total of 4 reciprocal translocations were observed in the treated males (1, 1 and 2 in the 15, 30 and 60 ppm groups, respectively) and none in the controls.

Although not subjected to sophisticated behavioral testing, none of the males demonstrated the hind limb splaying characteristic of acrylamide neuropathy. No significant pathological lesions of the sciatic nerve were noted under light microscopy for either the paraffin or plastic embedded tissue.

Discussion

The results reported here confirm the preliminary observations of Zenick et al. (1985) that acrylamide produces dominant lethality in the male rat. In the present study a cumulative intake of approximately 204 mg/kg of acrylamide, consumed at an average daily dose of 2.8 mg/kg was sufficient to produce significant elevation in post-implantation loss.

Preimplantation loss was significantly increased at 432 mg/kg or 5.8 mg/kg/day, though in this case some caution is necessary in interpreting the

TABLE 1

ACRYLAMIDE INTAKE AND ANIMAL WEIGHT AT BEGINNING OF MATING PERIOD (Means ± SEM)

Dose of acrylamide in drinking water (ppm)	Average weight at mating (g)	Average fluid consumption (ml/day)	Average dose acrylamide[a] (mg/kg/day)	Cumulative dose acrylamide at mating[b] (mg/kg)
0 ($N = 10$)	493.2 ± 17.3	43.2 ± 1.3	–	–
15 ($N = 10$)	503.5 ± 17.5	48.9 ± 2.6	1.5 ± 0.1	112.6 ± 7.4
30 ($N = 10$)	509.9 ± 13.8	44.9 ± 4.6	2.8 ± 0.3	204.2 ± 22.5
60 ($N = 11$)	478.2 ± 18.4	45.2 ± 2.7	5.8 ± 0.3	432.2 ± 24.4

[a]Daily dose acrylamide calculated as the mg-consumption difference between two weighing times divided by the number of days represented in that time period, and by the body weight at the second time.

[b]Cumulative dose of acrylamide calculated as the total consumption in mg divided by the body weight at initiation of mating.

data. Zenick et al. (1985) have shown that acrylamide (100 ppm) may induce hind limb incoordination in the male which can interfere with proper intromission and subsequent ejaculation. As an outcome, ejaculated sperm fail to reach the uterus. In the present study, however, none of the animals showed either overt clinical signs of neuropathy or the classical axonal lesions of a 'dying back' syndrome under light microscopy. This is consistent with other rodent studies in which cumulative subchronic and chronic acrylamide doses of up to 500 mg/kg failed to produce neurotoxicity (Fullerton and Barnes, 1966; McCollister et al., 1964). Indeed, the published literature does not appear to contain reports of toxicity at these doses to any other target organ in the rat using this treatment regimen. It seems likely, therefore, that the preimplantation losses observed here were also the result of direct damage to the sperm.

TABLE 2

FERTILITY RATES AND PREGNANCY OUTCOMES
(Means ± SEM)

Dose of acrylamide in drinking water (ppm)	Percent fertility rate[a]	Percent pre-implantation loss[b]	Percent post-implantation loss[c]
0 ($N=9$)[d]	87	10.4 ± 1.8[e]	5.7 ± 1.6[e]
15 ($N=9$)	76	9.3 ± 2.3[e]	7.2 ± 1.6[e]
30 ($N=10$)	95	12.2 ± 1.4[e]	13.3 ± 2.1[f]
60 ($N=11$)	80	25.1 ± 4.0[f]	36.7 ± 5.6[g]

[a] Fertility rate = $\frac{\text{Number of pregnant}}{\text{Number of mated}} \times 100$

[b] Pre-implantation loss = $\frac{\text{Number of corpora lutea} - \text{number of implants}}{\text{Number of corpora lutea}} \times 100$

[c] Post-implantation loss = $\frac{\text{Number of implants} - \text{number of fetuses}}{\text{Number of implants}} \times 100$

[d] N = The number of males/group mating with two females. Group means based upon averaging outcomes for the two females/male.

[e,f,g] Different superscripts, $P \leq 0.01$ Mann-Whitney U Test.

In the study of Zenick et al. (1985) male rats consuming 100 ppm of acrylamide for 8–10 weeks showed dominant lethal effects but exhibited no adverse changes in testicular pathology or in the generally measured parameters of semen quality (sperm concentration, morphology and motility). The potential for germ-cell damage by acrylamide has been reported by Shiraishi et al. (1978) who observed increased chromosomal aberrations in the spermatogonia and primary spermatocytes of mice receiving 500 ppm of acrylamide in the diet. Marked germ-cell atresia was also present, however, suggesting that these lesions were inconsistent with the production of sperm, and thus would not result in a dominant lethal effect. Carlson and Weaver (1985) have shown that orally administered acrylamide reaches the testes in the mouse and binds to nucleic acids and protein, presenting a potential mechanism for sperm damage.

In the present study, no indication of chromosome aberrations was apparent in males sacrificed at the termination of treatment. The observation 12 weeks later of 4 reciprocal translocations in spermatocytes derived from treated spermatogonial stem cells, is suggestive of an effect but not conclusive. We have conducted a preliminary study to determine if acute, high dose exposure to acrylamide could induce chromosome aberrations in differentiating spermatogonia. A single injection of 125 mg/kg (i.p.) of acrylamide was given to 6 mice (C3H × 101). Spermatogonial cell preparations were made 6 h after injection, following a 2-h injection of colchicine. This sampling time was chosen such that the cell population analyzed would have been in the S-phase at the time of treatment. A total of 100 metaphase cells were analyzed from the treatment group and from two untreated mice. No chromosome aberrations were observed.

Because of the use of acrylamide polymers as coagulant aids in the treatment of drinking water,

there is potential for cumulative human exposure to residual monomer on a large scale. Until now the focus of concern for human health hazard from this compound has been upon neurotoxicity. Since the reproductive effect reported here may represent in the rat a more sensitive toxic endpoint, some reassessment of acrylamide may be in order.

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References

Bull, R.J., M. Robinson, R.D. Laurie, G.D. Stoner, E. Greisiger, J.R. Meier and J.A. Stober (1984) Cacinogenic effects of acrylamide in Sencar and A/J mice, Cancer Res., 44, 107–111.

Bull, R.J., M. Robinson and J.A. Stober (1984a) Carcinogenic activity of acrylamide in the skin and lung of Swiss ICR mice, Cancer Lett., 24, 209–212.

Burek, J.D., R.R. Albee, J.E. Beyer, T.J. Bell, R.M. Carreon, D.C. Morden, C.E. Wade, E.A. Hermann and S.J. Gorzinski (1980) Subchronic toxicity of acrylamide administered to rats in the drinking water followed by up to 144 days of recovery, J. Environ. Pathol. Toxicol., 4, 157–182.

Carlson, G.P., and P.M. Weaver (1985) Distribution and binding of [^{14}C]acrylamide to macromolecules in Sencar and Balb/c mice following oral and topical administration, Toxicol. Appl. Pharmacol., 79, 307–313.

Fullerton, P.M., and J.M. Barnes (1966) Peripheral neuropathy in rats produced by acrylamide, Br. J. Ind. Med., 23, 210–221.

Hashimoto, K., and W.N. Aldridge (1970) Biochemical studies on acrylamide, a neurotoxic agent, Biochem. Pharmacol., 19, 2591–2664.

Hashimoto, K., J. Sakamoto and H. Tanii (1981) Neurotoxicity of acrylamide and related compounds and their effects on male gonads in mice, Arch. Toxicol., 47, 179–189.

Lijinsky, W., and A.W. Andrews (1980) Mutagenicity of vinyl compounds in *Salmonella typhimurium*, Teratogen. Carcinogen. and Mutagen., 1, 259–267.

McCollister, D.D., F. Oyen and V.K. Rowe (1964) Toxicology of acrylamide, Toxicol. Appl Pharmcol., 62, 342–345.

Meredith, R. (1969) A simple method for preparing meiotic chromosomes from mammalian testes, Chromosoma, 26, 254–258.

Shiraishi, Y. (1978) Chromosome aberrations induced by monomeric acrylamide in bone marrow and germ cells of mice, Mutation Res., 57, 313–324.

Tilson, H.A. (1981) The neurotoxicity of acrylamide: an overview. Neurobehav. Toxicol. Teratol., 3, 445–461.

Zenick, H., E. Hope and M.K. Smith (1985) Reproductive dysfunction associated with acrylamide treatment, Toxicologist, 5, 118 (A469).

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